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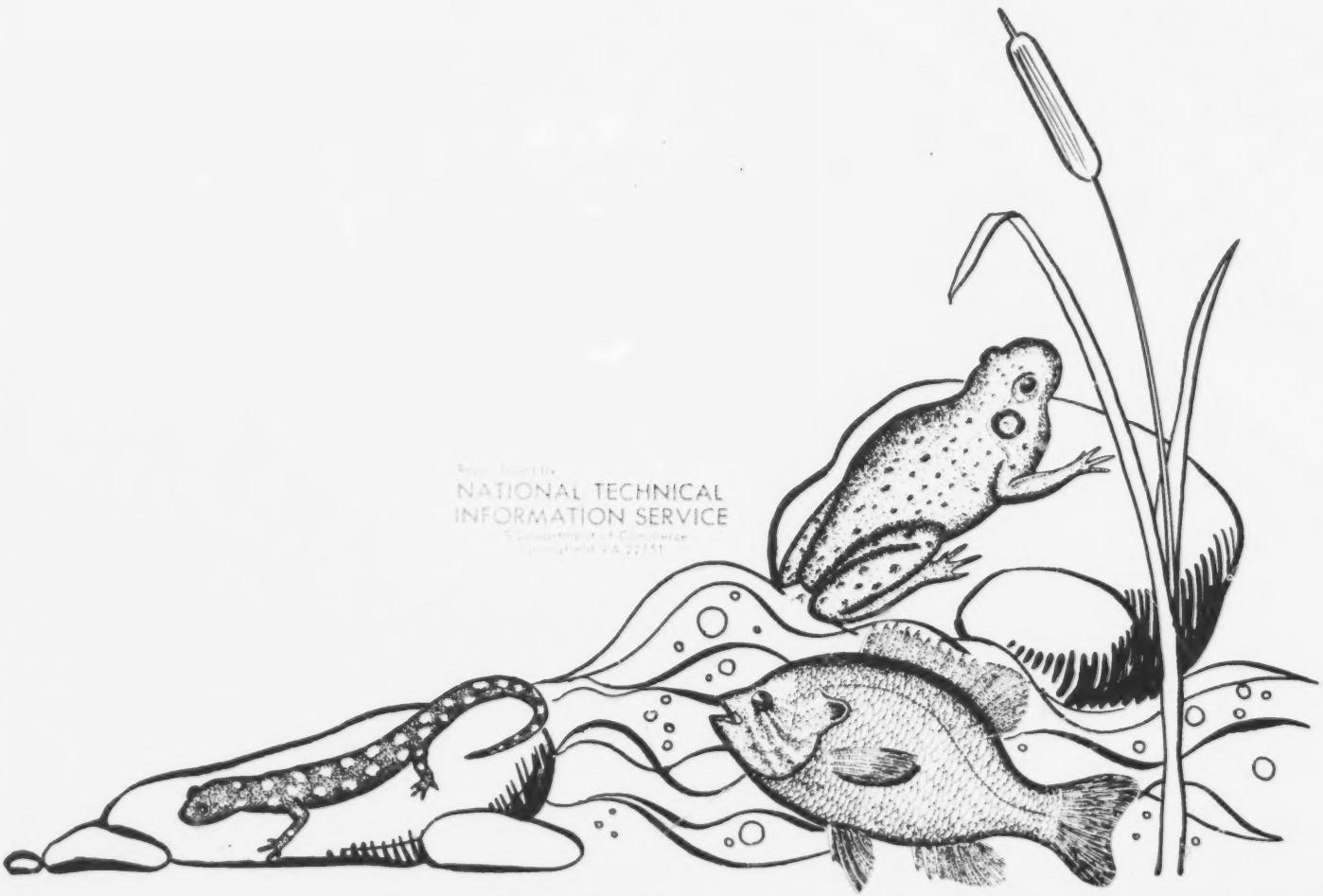

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THE USE OF BLUEGILLS TO DETECT ZINC

by

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for the

ENVIRONMENTAL PROTECTION AGENCY

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December 1971

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THE USE OF BLUEGILL BREATHING TO DETECT ZINC,

Cairns, J., Jr.; Sparks, R. E.

Project No. 18050 EDQ

Virginia Polytechnic Institute and State University, Blacksburg, Biology Department and Center for Environmental Studies

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The presence of zinc at concentrations of 8.7, 5.22, 4.16, and 2.55 mg/l in dechlorinated municipal tap water was detected by an increase in breathing rate or a change in breathing rate variance of bluegills. None of the fish exposed to the three lower concentrations died during the experiments. The criterion for detection was an arbitrary number of responses occurring at the same time. When the criterion was changed from a single response to three responses occurring at the same time, the number of false detections ('detections' occurring before zinc addition) decreased, but the lag between zinc addition and detection increased. Zinc concentrations of 0.025 and 0.075 mg/l (approximately 1/100 and 1/34 of 2.55 mg/l, respectively) did not appear to affect the reproduction and growth of bluegills in the laboratory, but 0.250 mg/l zinc (approximately 1/10 of 2.55 mg/l) inhibited spawning in ripe bluegills and killed newly-hatched fry. An in-plant system for the prevention of fishkills caused by spills could be developed by monitoring several biological functions of fish simultaneously to obtain informational redundancy and reduce error; by exposing test fish to higher waste concentrations than occur in the receiving stream as a safety factor; automating the collection and analysis of data to reduce lag time; and by choosing appropriate criteria for detection. (Mortland-Battelle)

*Zinc, *Bioindicators, *Water pollution effects, Sunfishes, Respiration, Toxicity, Lethal limit, Reproduction, Growth rates, Spawning, Monitoring, Industrial wastes, Bioassay, Sulfates, Fish physiology, Water pollution control, Water pollution sources, Heavy metals, Fishkill, Flow rates, Inhibitors,

*Bluegills, *Breathing, Biological samples, Zinc sulfate, Lepomis macrochirus

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John E. Mortland | Analytical Quality Control Lab., EPA, Cincinnati

ABSTRACT

The presence of zinc at concentrations of 8.7, 5.22, 4.16 and 2.55 mg/l in dechlorinated municipal tapwater was detected by an increase in breathing rate or a change in breathing rate variance of bluegills. None of the fish exposed to the three lower concentrations died during the experiments. The criterion for detection was an arbitrary number of responses occurring at the same time. When the criterion was changed from a single response to three responses occurring at the same time, the number of false detections ("detections" occurring before zinc addition) decreased, but the lag between zinc addition and detection increased.

Zinc concentrations of .025 and .075 mg/l (approximately 1/100 and 1/34 of 2.55 mg/l, respectively) did not appear to affect the reproduction and growth of bluegills in the laboratory, but .250 mg/l zinc (approximately 1/10 of 2.55 mg/l) inhibited spawning in ripe bluegills and killed newly-hatched fry.

An in-plant system for the prevention of fish kills caused by spills could be developed by monitoring several biological functions of fish simultaneously to obtain informational redundancy and reduce error; by exposing test fish to higher waste concentrations than occur in the receiving stream as a safety factor; automating the collection and analysis of data to reduce lag time; and by choosing appropriate criteria for detection.

This report was submitted in fulfillment of Project 18050 EDQ under the sponsorship of the Water Quality Office, Environmental Protection Agency.

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SECTION I

CONCLUSIONS

1. Breathing signals of bluegills show diurnal changes in rate and amplitude.

2. The presence of zinc at concentrations of 8.7, 5.22, 4.16 and 2.55 mg/l in dechlorinated municipal tapwater was detected by increases in breathing rate. The presence of zinc at a concentration of 4.16 mg/l was also detected by changes in breathing rate variance. Increasing the criterion for detection from a response by one fish to simultaneous responses by three fish generally reduced the number of false detections ("detections" occurring before zinc was added to the water) and increased the lag between introduction of zinc and detection.

3. Zinc concentrations of .075 and .025 mg/l (approximately 1/34 and 1/100 of 2.55 mg/l) in dechlorinated municipal tapwater did not appear to affect the reproduction and growth of bluegills in the laboratory, but spawning was markedly reduced and there was complete mortality of fry in .250 mg/l zinc (approximately 1/10 of 2.55 mg/l). A zinc concentration of .250 mg/l is not safe for chronic exposure of bluegills under conditions used in these experiments.

4. A workable biological monitoring system probably could be developed for the prevention of fish kills caused by industrial spills by monitoring two or more biological functions to increase informational redundancy and reduce error, automating data collection and analysis, exposing test fish to higher waste concentrations than occur in the receiving stream as a safety factor, and choosing appropriate criteria for detection.

SECTION II

RECOMMENDATIONS

The technique for monitoring fish breathing could be useful in the prevention of fish kills caused by industrial spills and as a source of biological information on which decisions in a river management system could be based.

We therefore recommend that a system for monitoring the response of fish to water quality be developed as follows: (1) by combining the technique for monitoring fish breathing with a technique for monitoring fish activity, already developed under Project 18050 EDP Water Quality Office, Environmental Protection Agency, thereby increasing the reliability of the monitoring system (2) reducing the time required for data analysis by converting analog breathing signals to numbers and then processing by computer (3) by laboratory testing of the system under simulated industrial conditions to determine whether water potentially toxic to fish can be detected fast enough to permit survival and recovery of the fish in the monitoring unit and fish maintained under simulated "downstream" conditions (4) subsequent testing of the monitoring unit at an actual industrial site.

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SECTION III

INTRODUCTION

The purpose of this research was to develop a method for rapidly detecting nonlethal effects of toxicants on the breathing rate of fish. In conjunction with already existing methods for measuring the chemical and physical characteristics of water, methods for rapidly measuring biological effects of water quality would be useful in industrial plants to prevent fish kills caused by spills, in river basins to provide information on which quality control measures could be based (Cairns, et al., 1970) and in water quality laboratories to provide the same information on a toxicant as a chronic bioassay, but in less time, as Drummond and Spoor (1971) have demonstrated.

With the above potential applications in mind, the research was directed to the goal of simplifying data collection and speeding analysis as much as possible.

This research was an extension of preliminary work on the effects of lethal concentrations of zinc sulfate on the breathing rate and heart rate of bluegills, Lepomis macrochirus Rafinesque (Cairns, et al., 1970). The electrocardiogram (ECG) was obtained by inserting a needle electrode in the breast of the fish. This technique had to be abandoned during the course of the present experiments because the tissue around the site of electrode insertion softened within two days, the electrode pulled out, and ECG's could not be obtained for the four to eleven day duration of the experiments.

For the purpose of developing a new technique, it seemed best to use a toxicant that was easily dosed and measured, and whose toxicity to fish was well documented: zinc sulfate. The test species was the bluegill.

The effects of zinc sulfate on the growth and reproduction of bluegills was also determined in an attempt to relate the effects of various zinc concentrations on the breathing rate to safe concentrations for chronic exposure.

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SECTION IV

EFFECTS OF ZINC ON FISH BREATHING

Materials and methods: Bluegills were obtained from the McKinney Lake National Fish Hatchery, Hoffman, North Carolina, and were held for at least two months in 50-gallon aquaria in a stock room prior to being used in an experiment. The mean weight of the 52 fish used in the experiments was 86.4 gms (S.D. 21.4) and the mean standard length (the distance from the tip of the snout to the origin of the caudal fin rays) was 13.4 cm (S.D. 1.1). Fish were fed frozen Gordon Formula daily (Axelrod, 1952), while the room lights were on, at a time chosen from a random number table, to keep from training the fish to feed at a particular time.

The stock room and the experimental room received water from a 500-gallon tank where the temperature was adjusted and the chlorine removed from municipal tap water by dripping in sodium thiosulfate. At least once a day, the following characteristics of the water were determined: phenolphthalein alkalinity, total alkalinity by the brom-cresol green-methyl red indicator method (Standard Methods, 1965), pH with a Fisher Accumet pH meter, total hardness with a Hach test kit, temperature with a mercury thermometer, and chlorine with a Hach model CN-46 chlorine tester. Dissolved oxygen concentrations were measured at random intervals by the azide modification of the iodometric method (Standard Methods, 1965), and copper and zinc concentrations were measured at random intervals by atomic absorption spectrophotometry.

Seven fish that were to be used in an experiment were netted from the stock tanks and placed in separate 5-gallon tanks in the experimental room for one week. Each fish was then moved into another 5-gallon tank in the same room for another week, before being used in an experiment. All the 5-gallon tanks were painted black on the outside so the fish could not see each other. By following this routine, experiments using six fish (one extra fish was kept as a spare in case of disease or injury) could be run once a week, while additional groups of seven fish were acclimating. Fish were fed approximately 1/4 gram of frozen Gordon Formula at a time chosen from a random number table once each day during the two-week additional acclimation period, and were not fed during experiments, to keep feces from accumulating in the test chambers.

The 8 x 8 x 8 foot experimental room was constructed to minimize disturbances to the fish, because preliminary experiments had shown that floor vibrations or people talking could cause the heart rate of the fish to slow and breathing to cease for several seconds. The sides and ceiling of each room were made of an outer wall of 1/2-inch plywood, a 3-inch inner layer of fiberglass insulation and an inner wall of 1/2-inch Celotex. There was a door in one wall. The table which held the test chambers rested on foam rubber pads and all electrical and water connections were flexible to minimize the transmission of vibrations.

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Durotest Optima fluorescent bulbs were used to approximate the spectrum of natural light. A dimming unit simulated dawn and dusk by turning on the lights and gradually increasing the intensity over a half-hour period starting at 6:30 a.m., and gradually decreasing the intensity to 0 over a half-hour period starting at 6:30 p.m. The maximum light intensity was approximately 100 foot candles, and the minimum intensity before the lights went out was approximately 1/2 foot candle, measured at the surface of the aquaria.

A Grass model 5-D polygraph was modified so that it would automatically make 15-minute recordings of breathing signals at half-hour intervals from 6 to 8 in the morning and evening and at one-hour intervals the rest of the day. Shielded cables running through the walls of the isolation room connected each test chamber to a polygraph channel.

The test chambers and the methods of preparing the fish were the same as described by Cairns, et al. (1970) for experiments with lethal zinc concentrations. The fish were placed in test chambers by 6 p.m. one day and the recording began at 6 a.m. the next day. The only changes were that six plastic test chambers were used at a time, and each chamber was placed in a compartment, open only at the top. Therefore, adjacent fish could not see each other, and if one became disturbed enough to swim, the response would not pass from one fish to another. Water or zinc solutions were pumped into the test chambers by Harvard variable speed peristaltic pumps (model number 1210). Water and test solutions flowed by gravity out of the test chambers and were not reused. It was necessary to insert a stopcock and a flow meter in each line to control the flow rates.

Zinc solutions were prepared by thoroughly dissolving a weighed amount of reagent grade $ZnSO_4 \cdot 7H_2O$ (Fisher catalog No. Z-68) in 95 liters of dechlorinated tap water drawn from the 500-gallon reservoir and stored in a plastic garbage can. The amount of $ZnSO_4 \cdot 7H_2O$ was calculated to yield a certain nominal concentration of Zn^{++}. Up to six garbage cans were connected by siphons. Fresh zinc solutions were prepared twice a day. The peristaltic pumps withdrew the solution from the last garbage can which contained a temperature controller. Actual zinc concentrations in the garbage cans and the outflow from each test chamber were determined daily by atomic absorption spectrophotometry, starting with experiment 6.

Table 1 shows the conditions for each of nine experiments. An electrode was inserted in each fish for some of the experiments. Breathing signals were obtained even when the electrode pulled out of the fish, so four experiments were carried out with the electrode taped to the bottom of the test chamber. Tricaine methanesulfonate (MS-222) at a concentration of 100 mg/l was used to anesthetize fish when the electrode was inserted, and no anesthetic was used in the other experiments.

No zinc was added to the water during the first four experiments, to determine whether there were diurnal patterns and day-to-day changes

Table 1 . Experimental conditions

Experiment	No. of control fish exposed to dilution water with no zinc added	No. of fish exposed to zinc	Duration of control period (prior to zinc addition)	Duration of exposure to zinc	Total duration	Active electrode in fish
1	6	0	96 hours	0 hours	96 hours	yes
2	6	0	146 hours	0 hours	146 hours	yes
3	6	0	98 hours	0 hours	98 hours	yes
4	6	0	120 hours	0 hours	120 hours	no
5	1	5	28 hours	96 hours	124 hours	no
6	1	5	28 hours	92 hours	120 hours	yes
7	1	5	28 hours	92 hours	120 hours	yes
8	2	4	148 hours	96 hours	244 hours	no
9	1	3	127 hours	48 hours	175 hours	no

Table 2. Method for analyzing the breathing rates of bluegills, experiment 8

Period			Dawn					Light		
Hour	6	6:30	7	7:30	8	9	10	11	12	1
Day 1										
Fish 1	27	30	39	42	(44)	42	40	42	39	41
Fish 2	29	20	29	(40)	34	34	32	28	28	28
Fish 3	11	12	15	(24)	19	(18)	18	15	18	18
Fish 4	11	11	16	(17)	16	(16)	13	16	14	14
Fish 5C	21	21	23	30	(36)	(39)	36	36	35	32
Day 2										
Fish 1	20	21	29	27	32	35	34	30	26	30
Fish 2			18	24	29	28	40	42	34	26
Fish 3	12	9	10	12	14	14	15	12	18	12
Fish 4	9	8	14	10	9	11	10	11	10	10
Fish 5C	17	18	19	26	33	30	37	33	33	31
Total	0	0	0	0	0	0	0	0	0	0
Day 7							↓			
Fish 1	19	20	12	18	16	22	24	26	15	28
Fish 2		16	28	31	32	34	34	36	24	27
Fish 3	14	16	17	16	14	16	[22]	20	16	15
Fish 4	11	9	8	10	11	10	11	12	16	16
Fish 5C	16	16	18	23	24	28	28	28	24	25
Total	0	0	0	0	0	0	1	0	0	0

Note: Blanks indicate that the amplitude of the breathing signal was so low that the rate could not be determined.

↓ Measured zinc concentration of 4.16 mg/l introduced, excent for Fish 5, which was not exposed to zinc.

(◯) Maximum breathing rate for each fish during each period of the first day.

[□] Breathing rates on second and seventh days which exceeded first day maxima. The total number of fish showing increased breathing rates is shown at the bottom of each column.

Table 2 (continued)

2	3	4	5	6	6:30	7	7:30	8	9	10	11	12	1	2	3	4	5
				Dusk											Dark		
									Day 1								
37	38	(44)	35	38	45	(57)	25	23	20	22	20	21	25	24	24	26	(27)
27	46	40	(49)	(49)	40	27	24	16	12	(15)							14
16	15	16	14	13	13	16	(18)	10	9	11	10	10	9	12	8	12	(13)
12	11	12	12	(13)	13	13	12	9	9	8	10	10	7	8	8	10	(11)
32	35	32	33	(43)	42	37	27	21	19	20	18	19	18	16	(21)	19	16
									Day 2								
27	22	33	22	24	31	22	15	15	16	17	14	15	13	15	16	13	16
30	24	45	42	46	22	19	28	24	15			[18]			[19]	15	15
12	13	13	15	16	16	13	18	11	10	10	10	11	12	13	10	10	10
10	10	10	13	12	13	10	12	8	7	7	8	8	9	8	8	10	10
30	32	27	31	36	28	29	23	22	21	17	16	16	20	16	17	18	21
0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
									Day 7								
[62]	22	26	34	30	38	30	57	52	[50	48	48	46	47	43	48	49	46]
28	28	20	46	40	40	42	[64]	18	[20	22	28	23]					
12	13	15	14	15	16	15	13	10	[16]	12	[14]	9	[14	16	14]	9	12
16	11	11	10	11	10	12	11	10		[62	61	59	55	49	59	54	55]
30	27	26	26	29	28	26	11	15	17	16	14	14	15	18	15	15	15
1	0	0	0	0	0	0	1	0	3	3	4	3	3	3	3	2	2

in the breathing rate. The results showed that analysis of variance techniques could not be used for statistical comparisons between experiments with no added zinc and later experiments with zinc because the breathing rate variances at different times of day for the same fish, and at the same time of day for different fish, were heterogeneous.

An alternative method of analyzing the data was based on the observed diurnal change in breathing rate (Sparks, et al., 1970), and on the observation that the breathing rate at each hour during the first day of recording was generally greater than the breathing rate at the same hour on subsequent days, perhaps due to incomplete recovery from the stress of handling. The experimental day was divided into four periods: (1) a period from 6 to 8 a.m. (dawn), when the breathing rate changed markedly (2) a period from 9 a.m. to 5 p.m. (light) when the rate was comparatively high (3) another period of rapid change from 6 to 8 p.m. (dusk) and (4) a period from 9 p.m. to 5 a.m. (dark) when the rate was comparatively low. The maximum breathing rate of each fish during each period of the first day was circled, as in Table 2. Any increase in a fish's breathing rate during a subsequent period that exceeded the maximum rate observed during the corresponding period of the first day was considered to be a response to a stimulus greater than the stimulus of being netted and placed in the test chamber. A box is drawn around the responses in Table 2 that occurred on day 2, before any zinc was added to the water, and on day 7, when zinc was added at 10 a.m. The total number of experimental fish (fish exposed to zinc on day 7) showing responses at each hour of day 2 and day 7 is shown. The control fish, 5C, was not exposed to zinc and showed no responses. There are missing observations in Table 2 because of a diurnal breathing amplitude change. The amplitude of the breathing signals of some fish was so low that rates during the dark and dawn periods could not be determined every hour. Maximum breathing rates during each period of the first day were determined from whatever values were available. If no breathing rates could be determined for a fish during a whole period of the first day, no further analyses were made using that fish.

The results also suggested another method of analyzing the data. During the four periods of the day described above, the breathing rate of a fish exposed for several hours to a sublethal concentration of zinc generally would fluctuate between high values and the normal values for that fish. The fluctuation in breathing rate could be expressed quantitatively as a variance, and a two-sample test for heterogeneity of variances was used to determine whether a significant difference existed between breathing rate variances (Sokal and Rohlf, 1969). The breathing rate variance for each period of the first day was computed for each fish, and compared to the variance for the corresponding period of the second day for the same fish. If the variances were not significantly different, then the fish was considered to be exhibiting normal variation in breathing rate on day 2, and the variance for each period of day 2 then was compared to the variance for the corresponding period of day 3. If the variances for corresponding periods of days 1 and 2 were significantly different,

the fish was considered to be exhibiting a response on day 2, the variance for that period of day 2 was dropped, and the next comparison was made between corresponding periods of day 1 and day 3. For example, if the breathing rate variance for fish 1 during the dawn period of day 1 was significantly different from the variance during the dawn period of day 2, the fish was considered to have shown a response during the dawn period of day 2. The variance for the dawn period, day 2, was dropped, and the breathing rate variance for the dawn period of day 3 was compared to the breathing rate variance for the dawn period of day 1. Experiment 8 was analyzed both by comparing breathing rates on each day to the maximal rates on day 1 and by variance comparisons. The effect of using two significance levels ($\alpha = .05$, $\alpha = .01$) on the number of responses obtained by variance comparisons was determined.

As stated above, the response of an individual fish was defined as a change in breathing rate variance or an increase in breathing rate. The number of fish showing responses at one time was used as the criterion for detection of zinc, and the effect of requiring different numbers of simultaneous responses was examined. For example, experiment 8 was analyzed using the rule that a response by a single fish would count as a zinc detection. The number of false detections ("detections" occurring before any zinc was added to the water) and the lag time (the time between zinc addition and detection) were determined. Experiment 8 was analyzed again using the rule that responses by two fish at the same time would count as a detection. The number of simultaneous responses required for a detection was increased up to the total number of fish exposed to zinc (three or four), and the number of false detections and the lag time determined in each case.

Unavoidably, various extraneous disturbances to the fish were included in the experiments. The door of the experimental room was opened for three minutes once a day during each experiment in order to feed the fish that were acclimating. The only response by the fish was a pause in breathing lasting up to half a minute, followed by rapid breathing for a few more minutes. Breathing rates were never taken from the disturbed portion of the record. An earthquake of sufficient magnitude to rattle bookcases occurred during experiment 1. Workmen spent two hours breaking open a cement wall in an adjoining laboratory during experiment 9. The effects of the last two disburbances are discussed in the next section.

Results: The characteristics of the dilution water are shown in Table 3 and the flow rates and zinc concentrations for each experiment are shown in Table 4.

One of five fish exposed to a zinc concentration of 8.7 mg/l died after 77 hours of exposure and another died after 87 hours. None of the fish died during any of the other experiments described below. Each of the fish exposed to 8.7 mg/l zinc showed increases in breathing rate after 5-16 hours of exposure. Maximum breathing rate increases ranged from

Table 3 . Routinely determined characteristics of dilution water.

Water Characteristic	Number of Analyses	Mean	Standard Deviation
Total hardness (mg/l as $CaCO_3$)	394	51	10
Phenolphthalein alkalinity (mg/l as $CaCO_3$)	393	0.0	0.0
Total alkalinity (mg/l as $CaCO_3$)	393	41.3	8.8
pH	397	7.8	0.3
Temperature (^{o}C)	396	19.7	1.8
Dissolved oxygen	Maintained at air saturation		
Chlorine	None		
Zinc, copper	Zinc and copper concentrations were less than .03 mg/l in random analyses of the dilution water by atomic absorption photospectrometry.		

Table 4. Flow rates and zinc concentrations during experiments.

Experiment	Nominal zinc concentration (mg/l)	Measured concentration (mg/l) mean	S. D.	No. of samples	Flow rates (ml/min) mean	S. D.	No. of samples
1	0	----	----	--	105	7	42
2	0	----	----	--	100	3	42
3	0	----	----	--	98	5	18
4	0	----	----	--	97	10	36
5	8.7	----	----	--	98	7	72
6	4.9	4.28	0.25	20	94	7	42
7	2.8	2.55	0.42	15	84	12	36
8	4.9	4.16	0.27	24	98	5	54
9	6.0	5.22	0.20	9	99	2	40

Note: Dashes indicate that no analyses were made.

51-73 breaths/min. and occurred after exposure times ranging from 5 to 79 hours. Further analysis was impossible because of inadequate records.

Table 5 shows the breathing rate response data for three fish exposed to a measured zinc concentration of 5.22 mg/l and one control fish exposed at the same time to dilution water containing no added zinc. The breathing rates of the control fish exceeded the first-day maximal rates just once (12 midnight, day 6). During the control period, before any zinc was added, there was only one occasion when two experimental fish showed a response at the same time(1 a.m., day 3). On 11 other occasions during the control period, a single fish showed a response (not the same fish every time), but two of these responses were probably due to sustained pounding by workmen who were breaking open a cement wall in a room next to the laboratory. In contrast, after the zinc was introduced, there were seven times when two fish showed a response simultaneously and 26 times when a single fish showed a response.

Table 6 shows the responses of four fish exposed to a measured zinc concentration of 4.16 mg/l and one control fish. The control fish showed a response just once (9 p.m., day 4). During the control period there were 15 occasions when a single experimental fish responded, and three occasions when two experimental fish responded at the same time. At no time during the control period did more than two fish show responses together. After the zinc was introduced, all four of the exposed fish showed responses simultaneously on five occasions, and three fish showed responses during the same time interval on 19 occasions. If the criterion for detection of water conditions potentially harmful to fish were two or more responses during the same time period, then three false detections would have occurred before any zinc was added, and 4.16 mg/l zinc would have been correctly detected 8 hours after it was introduced. If the detection criterion were three or more responses during the same time period, then no false detections would have occurred and the zinc would still have been correctly detected after 8 hours.

Analysis of experiment 8 by variance comparisons also indicated a marked response by the fish to 4.16 mg/l zinc (Table 7). With $\alpha = .05$, no more than two fish at a time showed responses during the control period, whereas three fish at a time showed responses after zinc was added. With $\alpha = .01$, no more than one fish at a time showed a response during the control period, whereas two and three fish responded at a time after the zinc was added.

Table 8 shows the results obtained from three fish exposed to the lowest zinc concentration tested, 2.55 mg/l, and from six control fish (from experiment 1) that were exposed to dilution water containing no added zinc. Only one fish at a time out of the six control fish ever showed a response. The first occasion when two of the experimental fish showed responses at the same time was 8 hours after the zinc was introduced. Three experimental fish showed responses at the same time 52 hours after

Table 5. Number of fish showing responses, before and after exposure to 5.22 mg/l zinc

Day		Time																						
		6am	7	8	9	10	11	12	1pm	2	3	4	5	6	7	8	9	10	11	12	1am	2	3	4	5
2	Ex	0	0	0	0	0	0	0	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0
	Con	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	Ex	0	0	0	0	1*	1*	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0
	Con	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	Ex	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
	Con	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	Ex	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0
	Con	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	Ex	0	0	0	0	0	↓1	0	0	0	2	2	0	1	0	1	0	1	1	1	1	1	1	1	1
	Con	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
7	Ex	1	0	0	0	1	2	1	1	1	1	1	2	1	2	0	1	1	2	1	1	1	1	2	0
	Con	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	Ex	0	1	0	end of experiment																				
	Con	0	0	0																					

*Workmen were breaking open a cement wall

↓Measured zinc concentration of 5.22 mg/l introduced. Responses obtained during zinc exposure are underlined.

Note: There were 3 experimental fish (Ex) from Experiment 9 and 1 control fish (Con) from Experiment 9.

Table 6 . Number of fish showing responses, before and after exposure to 4.16 mg/l zinc.

	Time																							
Day	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5
	am							pm												am				
2																								
Ex	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
Con	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3																								
Ex	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
Con	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4																								
Ex	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	Recorder			off-		-	-	-
Con	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-	-	-		-	-	-	-
5																								
Ex	-	-	-	0	0	0	0	0	0	1	0	0	1	0	0	1	1	0	0	2	1	1	1	0
Con	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6																								
Ex	Recorder		0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	2	0	1	2
	Off-	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

NOTE: There were 4 experimental fish (Ex) from Experiment 8 and 1 control fish (Con) from Experiment 8.

Table 6 . (continued)

	Time																							
Day	6 am	7	8	9	10	11	12	1 pm	2	3	4	5	6	7	8	9	10	11	12	1 am	2	3	4	5
7																								
Ex	0	0	0	0	↓1	0	0	0	1	0	0	0	0	0	0	3	3	4	3	3	3	3	2	2
Con	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8																								
Ex	2	0	0	1	0	2	0	0	1	1	1	2	1	1	0	4	4	3	3	4	3	4	3	1
Con	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9																								
Ex	0	0	1	1	2	1	3	1	1	2	1	1	1	2	2	2	3	3	3	3	3	2	2	2
Con	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10																								
Ex	0	2	0	0	0	0	0	0	1	0	0	1	0	0	1	2	3	2	2	3	3	1	2	2
Con	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11																								
Ex	0	0	0	0	0	end of experiment																		
Con	0	0	0	0	0																			

↓ Measured zinc concentration of 4.16 mg/l introduced. Responses obtained during zinc exposure are underlined.

Table 7. Number of fish showing heterogeneous variances, before and after exposure to 4.16 mg/l zinc.

Day		α = .05 Dawn	Light	Dusk	Dark	α = .01 Dawn	Light	Dusk	Dark
2	Ex	0	0	0	0	0	0	0	0
	Con	0	0	0	0	0	0	0	0
3	Ex	0	0	1	2	0	0	1	1
	Con	0	0	0	0	0	0	0	0
4	Ex	0	1	2	1	0	1	0	0
	Con	0	0	0	0	0	0	0	0
5	Ex	-	0	0	1	-	0	0	0
	Con	-	0	0	0	-	0	0	0
6	Ex	0	1	0	1	0	1	0	0
	Con	0	0	0	0	0	0	0	0
7	Ex	0	↓2	1	2	0	↓2	0	0
	Con	0	0	0	0	0	0	0	0
8	Ex	2	1	2	3	1	1	1	1
	Con	0	0	0	0	0	0	0	0
9	Ex	0	3	1	2	0	2	0	2
	Con	0	0	0	0	0	0	0	0
10	Ex	2	3	1	2	0	3	0	1
	Con	0	0	0	0	0	0	0	0
11	Ex	0				0			
	Con	0				0			

↓ Measured zinc concentration of 4.16 introduced
Responses obtained during zinc exposure are underlined.

Note: There were 4 experimental fish (Ex) and 1 control fish (Con).

the zinc was added and on two more occasions before the experiment was terminated 67 hours after zinc addition.

When an earthquake occurred at 8 p.m. during day 2 of experiment 1 (Table 8), the fish ceased breathing for 12 to 28 seconds. After approximately five minutes the breathing rates became normal again, and therefore no responses were recorded for experiment 1 at 8 p.m. in Table 8. Bluegills react similarly when the room lights are turned off abruptly or someone rapidly approaches the test chambers.

Tables 9 and 10 summarize information that indicates the effectiveness of the two methods of analysis. The tables show that changing the criterion for detection from one to three responses per time period generally increases the lag time and decreases the number of false detections. Table 10 also shows that there are fewer false detections by the variance comparison method with $\alpha = .01$ than with $\alpha = .05$. In addition, the lag time was no greater for $\alpha = .01$ than for $\alpha = .05$ when either one or two responses per time period counted as a detection.

If three simultaneous breathing rate increases counted as a detection, Table 9 shows that no false detections would occur in the three experiments reported, but that the highest zinc concentration (5.22 mg/l) would not have been detected before experiment 9 ended. The effect of missing observations on the results would be to underestimate the number of responses occurring at a time, and although the diurnal decrease in breathing amplitude resulted in missing observations during the dawn and dark periods for approximately one out of five fish, there were no missing observations for the fish used in experiment 9. As only three fish were exposed to zinc in this experiment, it is possible that one unusually zinc-tolerant individual determined the outcome of the test for detection.

If the criterion for detection in experiment 8 were two simultaneous responses, then no false detections would have been obtained by the variance comparison method with $\alpha = .01$ (Table 10), whereas three false detections would have been obtained by measuring increases in breathing rates (Table 9). In addition, the lag time would be less using the variance comparison method.

Discussion: Of the two methods used to analyze fish breathing rates, checking for increases in breathing rate is the most attractive because it permits a response test as each successive value is generated. The first day, of course, must be used to obtain the maximal breathing rates used in all subsequent comparisons. The variance comparison method at first appears limited because enough values must accumulate to obtain estimates of variances. For example, if zinc had been introduced at the beginning of the light period of day 7, experiment 8 (Table 7), it would not have been possible in the present system to detect the zinc until 5 p.m., when nine values had accumulated, and the variance was computed and tested against the variance of the previous day. However,

Table 8 . Number of fish showing responses, before and after exposure to 2.55 mg/l zinc.

	Time																							
Day	6 am	7	8	9	10	11	12	1 pm	2	3	4	5	6	7	8	9	10	11	12	1 am	2	3	4	5
2																								
Ex	0	0	1	1	1	1	1	1	1	1	1	1	2	1	2	1	1	1	1	0	1	1	1	1
Con	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0*	0	1	0	0	0	0	0	0	0
3																								
Ex	1	2	1	1	1	1	1	1	1	2	1	1	1	1	0	1	0	0	0	1	1	2	1	--
Con	0	0	0	1	1	1	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	1	0
4																								
Ex	recorder off			1	1	0	0	2	3	3	2	1	0	0	1	1	2	1	2	2	2	2	2	3
Con	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0

↓ Measured zinc concentration of 2.55 mg/l introduced. Responses obtained during zinc exposure are underlined.

Note: There were 3 experimental fish (Ex) from Experiment 7 and 6 control fish (Con) from Experiment 1.

* Earthquake occurred during the 8 p.m. recording, day 2, Experiment 1.

Table 9 . Effectiveness of zinc detection using increases in fish breathing rates

Zinc (mg/l)	No. of fish Exposed to zinc	Detection criterion: minimum no. of fish showing response at one time	Lag time (Hrs. from addition of zinc)	False detections
5.22	3	1	0	12 in 100 hrs.
		2	4	1 in 100 hrs.
		3	not detected after 45 hrs.	0 in 100 hrs.
4.16	4	1	0	19 in 123 hrs.
		2	11	3 in 123 hrs.
		3	11	0 in 123 hrs.
2.55	3	1	0	2 in 4 hrs.
		2	8	0 in 4 hrs.
		3	52	0 in 4 hrs.

Table 10. Effectiveness of zinc detection using successive comparisons of breathing rate variances.

Zinc (mg/l)	No. Fish	Detection criterion: minimum no. of fish showing heterogeneous variances during one time period $\alpha = .05$	Lag time (Hrs. from addition of zinc)	False detections
4.16	4	1	7	8 in 122 hrs.
		2	7	2 in 122 hrs.
		3	43	0 in 122 hrs.
		Detection criterion: minimum no. of fish showing heterogeneous variances during one time period $\alpha = .01$		
		1	7	4 in 122 hrs.
		2	7	0 in 122 hrs.
		3	79	0 in 122 hrs.

breathing rates could be taken every minute and the variances estimated and tested every 10 minutes by means of analog-to-digital converters, mini-computers, and teleprinters. As swimming masks the breathing signals, the computer would have to be programmed to distinguish breathing from swimming. In addition, the amplification of the breathing signal might be controlled to compensate for the diurnal fluctuation in signal amplitude. The system would be relatively immune to sudden extraneous disturbances (such as floor vibrations or a loud noise) if the computer were programmed to skip the transitory pauses in the breathing signal that such disturbances produce.

One advantage of the method of variance comparisons is that a decrease in breathing rate variance, as well as an increase, counts as a response because the statistical test detects differences in variance, not the direction of difference. It is possible that some toxicants would reduce breathing rate variance. The disadvantage of counting breathing rate increases as responses is that some toxicants might act as respiratory depressants. This objection could be overcome by establishing minimal, as well as maximal breathing rates for each fish before the fish were exposed to toxicants, and then checking for values which fall outside this range on subsequent days. The method of choice could be determined by further testing of the breathing monitoring system with a variety of toxicants, singly and in combination.

The results show that the criterion for detection should be simultaneous responses by some proportion of the exposed fish, not by all of them, to reduce the probability of having the outcome determined by an exceptionally unresponsive fish.

It would certainly be desirable to have a redundant detection system in an industrial situation, where water and waste qualities are apt to vary unpredictably. It is conceivable that some harmful combination of environmental conditions and waste quality would be detected by monitoring one biological function but not by monitoring another. The present system for monitoring fish breathing could be combined with another system for monitoring fish movement (already developed as project 18050 EDP, Water Quality Office, Environmental Protection Agency) by using test chambers that permit breathing signals to be obtained from free-swimming fish (Spoor, et al., 1971). It should be possible to feed the test fish and use them as long as they live and do not become incapable of response after long exposure to toxicants, due to impairment of sensory mechanisms or development of resistance. It would be desirable to have some control fish exposed to upstream water containing no waste from the plant, in order to evaluate the effects of upstream conditions on the fish, and to detect extraneous effects such as floor vibrations or noises that continue for several hours and disturb the fish (Figure 1). The lag time inherent in the technological portion of the system could be reduced by automating data collection and analysis as described above and the lag time inherent in the biological portion of the system probably could be reduced by metering proportionally more waste into the dilution water delivered to the test fish than is delivered to the stream. The latter method would also serve to introduce a safety factor.

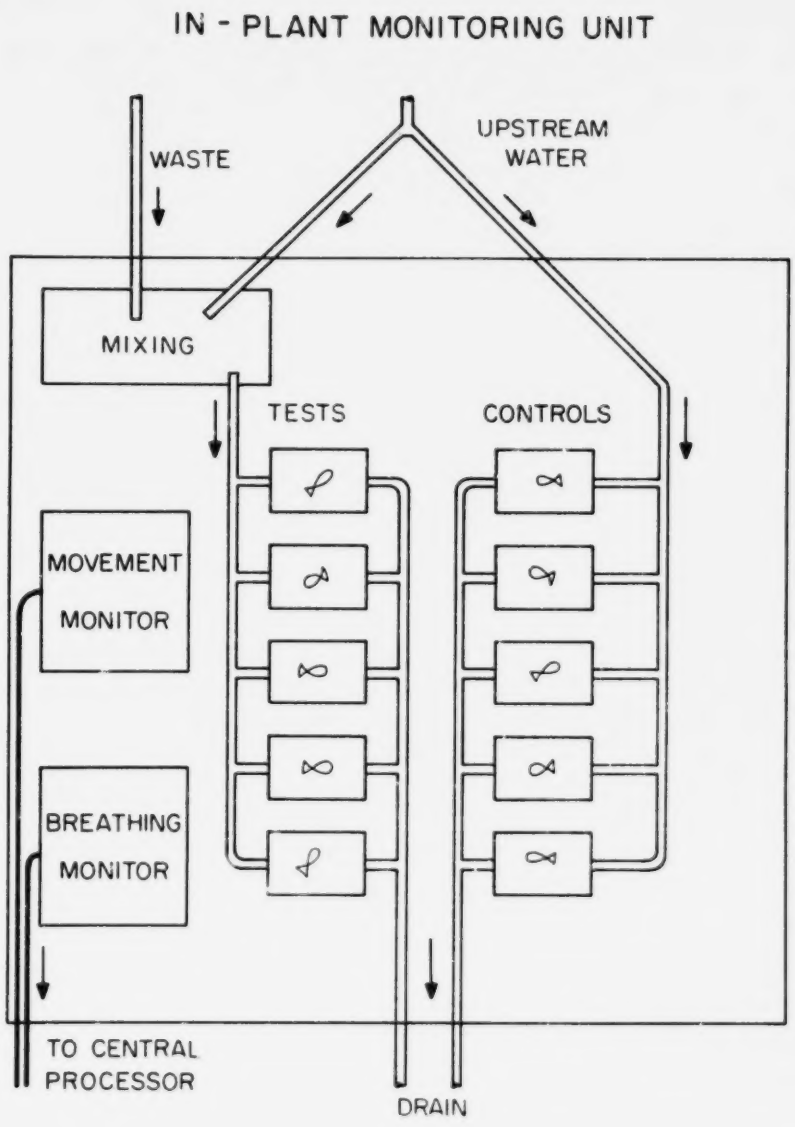

Figure 1. A monitoring unit for industrial use, showing how the test fish are exposed to waste diluted with upstream water and the control fish are exposed to upstream water alone.

If industrial users of a river complied with water quality standards and also were able to guard against accidental spills or a detrimental change in environmental conditions by means of a biological monitoring system, then full use could be made of a river without damaging the fish life.

SECTION V

FISH REPRODUCTION AND GROWTH

Methods and materials: Bluegill sunfish were seined from a local pond and held for several months in the laboratory in the same dilution water, with the same photoperiod and water temperatures, as for the experiments described in Section IV.

Starting April 13, approximately 200 fish (approximate total lengths: 8-15 cm; weights: 10-80 gms.) were brought into breeding condition by exposing them to a photoperiod of 16 1/2 hours of light, water temperatures of 31-32°C, and by feeding them twice daily with frozen Gordon Formula (Axelrod, 1952) and once daily with live mealworms. The dimming system described earlier simulated a 1/2 hour dawn starting at 6 a.m. and a 1/2 hour dusk starting at 10 p.m.

On May 4 most of the fish could be sexed by gently squeezing the sides and observing whether eggs or milt was extruded, and three females and one male were placed in each of twenty 20-gallon tanks (standard aquaria, long type, Ramfab Aquarium Products cat. no. RA-20L).

One standard clay flowerpot (upper rim-to-rim diameter = 6 inches) was placed on its side in each tank for the females to hide from the aggressive attacks of the males. An artificial nest, described by Eaton (1970) was also placed in each tank and five smooth pebbles, 2-3 cm in diameter, were scattered on the bottom of each nest.

One toxicant delivery apparatus was used for each set of five tanks receiving one concentration, and one water delivery apparatus was used for five control tanks which received no added zinc. The toxicant delivery apparatus combined a toxicant dipper and needle valve described by Mount and Brungs (1967) with a water delivery system described by Brungs and Mount (1970). The dilution water was the same as that used in the experiments described in Section IV. The zinc concentrations for the reproduction study were based on the lowest concentration used in the fish breathing experiments; i.e. 2.5 mg/l. Tanks 6-10 received .250 mg/l zinc (1/10 of 2.5 mg/l), tanks 16-20 received .025 mg/l zinc (1/100 of 2.5), and tanks 11-15 received no added zinc and served as controls. In addition, tanks 1-5 received 1/100 the 96-hour TL50 (median tolerance limit) for adult bluegill sunfish exposed to zinc in municipal tapwater: .075 mg/l. The initial zinc concentration used in the stock jug of each toxicant delivery apparatus was calculated to yield the desired concentration in the tanks. The concentrations in the tanks were measured by atomic absorption spectrophotometry and were lower than desired, so the concentrations of the stock solutions were adjusted until the correct concentrations were obtained in the tanks.

The flow rate to each tank was approximately 100 ml/min. The water entered at the top and front of the tank and was removed from the bottom

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carrying some detritus with it, by means of a sheathed standpipe at the rear of the tank.

A plastic egg hatching box (20.5 cm long, 7.0 cm wide, and 15.5 cm deep) hung on the front of each aquarium and was large enough to accept three egg cups. The egg cups were made from Turtox plastic jars (5.5 cm o.d., 6.8 cm tall), with the bottoms removed. In use, each end of the cup was covered with a piece of ladies' woven nylon hose held in place with rubber banks. Each cup rested on an airstone cemented to the bottom of the hatching box. A piece of plexiglas (20 cm long, 6.3 cm wide, and 0.6 cm thick) fit into grooves in the hatching box and rested on top of the cups to keep them from floating.

Water siphoned from each tank into the hatching boxes and was returned to the tank by an air lift. Another air lift delivered water from the hatching box to a plastic rearing chamber (38.0 cm long, 30.5 cm wide, and 17.5 cm deep) for rearing newly-hatched fry. Thus the eggs were hatched and the fry reared in the same water as their parents. Water drained from each rearing chamber through a rectangular opening (8.8 cm wide, 1.8 cm high) into a trough. The bottom of the opening was 14.5 cm from the bottom of the pan, and the opening was covered by .8 mm mesh nylon netting.

The fish in each tank were fed two grams of frozen Gordon Formula (Axelrod, 1952) twice a day and eight live mealworms once a day. The tanks were cleaned once a week by siphoning detritus from the bottom.

At 1 p.m. every day, the pebbles in each nest were removed and examined closely for eggs. If eggs were present, a plastic chamber (same dimensions as above) was filled with water from the tank and the nest was removed from the tank and placed upside down in the chamber over an airstone. A new nest was substituted immediately for the old one.

A subsample of 200 eggs was removed from the nest and placed in a hatching cup, which in turn was placed in the hatching box. After 48 hours, the number of fry in both the egg cup and the chamber were counted, by pipetting them into petri dishes and using a Dazor model M209 fluorescent magnifier and a hand tally counter. The hatch in the subsample of eggs in the cup was assumed to be proportional to the hatch in the nest, and the numbers of fry and eggs in the cup were used to back-calculate the number of eggs spawned in the nest:

$$\text{Total No. Eggs In Nest} = \text{No. Fry In Nest} \times (\text{No. Eggs in Cup} \div \text{No. Fry in Cup}) + \text{No. Eggs In Cup}$$

When less than 200 eggs were spawned, the number of eggs in the nest and the number of fry in the hatching chamber were counted directly, without removing a subsample.

Fish that were dead or that had lost their equilibrium were removed as soon as they were noticed. In addition, six fish had an eye disease that

started as a white spot and gradually consumed the entire eye, and these fish were also removed. Fish that were removed before the end of the experiment were weighed, measured and sexed--unless they were too decomposed. Fish that were removed and could be sexed were replaced by a fish of the same sex from a stock of ripe fish kept in dechlorinated tapwater containing no added zinc, until July 20, when no further replacements were made. The breeding portion of the experiment terminated August 19, when all the remaining adult fish were killed, weighed, measured and sexed. The condition and weight of the gonads was also recorded.

Fifty fry from the first spawning in each tank were placed in the rearing chamber for that tank. Newly-hatched brine shrimp were rinsed in dechlorinated tapwater and placed in each rearing chamber twice a day for the fish to feed upon.

A census of the rearing chambers on July 8 revealed that very few fish were surviving, so changes were made in the apparatus and methods. Some of the fry may have washed through the netting of the rearing chambers, so the chambers were modified by drilling five 1.1 cm holes on centers 11.5 cm above the bottom of the chambers and covering them with a piece of woven nylon. All surviving bluegills were transferred to the modified chambers on July 8 and 9, and all fry hatched after July 9 were placed in chambers of the new design.

In addition, we learned that brine shrimp were too large for bluegill fry and that the National Water Quality Laboratory, Duluth, Minnesota, was successfully feeding plankton to baby bluegills (James M. McKim, III, personal communication). Consequently, starting July 23, plankton was obtained regularly from nearby ponds, and fed, after straining through a 0.8 mm mesh net, twice a day to the fish. Samples of the plankton were examined regularly under the microscope and never appeared to be very rich, so the diet was supplemented with a pinch of TetraMin powdered baby fish food twice a day. Newly-hatched brine shrimp were fed to the fish starting approximately the third week of growth.

Since no spawnings ever occurred in some tanks, the rearing chambers for these tanks received fry from other tanks. In addition to transfers of fry made within chambers at the same zinc concentration, some fry were taken from high zinc concentrations and put into chambers containing low concentrations, and vice-versa.

The fry in each chamber were counted and total lengths determined 30, 60 and 90 days after introduction to the rearing chamber. Total lengths were determined by placing each fish in a glass petri dish over a metric ruler.

Dissolved oxygen concentrations in the breeding tanks were determined by a YSI oxygen meter, and temperatures by a mercury thermometer. Since water from the breeding tanks was delivered directly to the rearing chambers by air lifts, we assumed that the water characteristics in the

tanks and chambers were the same. This assumption was confirmed for zinc by measuring zinc concentrations in the tanks and chambers at random intervals, but D.O. and temperature were not measured in the rearing chambers.

Results and discussion: Zinc concentrations, dissolved oxygen concentrations (D.O.), and temperatures in the breeding tanks are shown in Table 11. On August 4, 1971, a new central air conditioning system began operating, and the room temperature was lowered 5.6°C in 1[illegible] hours. As a result, the temperatures in the breeding tanks reached a new steady state, approximately 4°C lower than the mean temperatures recorded earlier. Since a spawning occurred for the first time in tank 9 after the drop in temperature, and six additional spawnings occurred in other tanks, the temperature drop did not seem to affect spawning. However, eggs from a spawning on August 4 that were exposed to a drop in water temperature of 12°C showed a very low percentage hatch (2%) and were not included in the results.

The dechlorinated tapwater delivered to the control tanks (tanks 11-15) contained a zinc concentration ranging from .002 - .062 mg/l. The mean zinc concentrations and the standard deviations in all the tanks are shown in Table 11. Nominal zinc concentrations will be used in the rest of the text.

Data on the adult bluegills used as breeders are shown in Table 12. Although males and females were approximately the same size when they were introduced to the breeding tanks on May 4, Table 12 shows that the surviving males were generally heavier and longer than the surviving females when the breeding portion of the experiment terminated August 19. Also, mortality among females was proportionally greater than it was among males. The disparity in growth and survival between males and females was probably due to biting and butting attacks by the males. Most of the dead females had tattered fins and scales missing from their sides. Females that were in tanks with very aggressive males would feed hesitantly, even when food was placed near their flowerpot shelters. Most of the fish at all zinc concentrations were still ripe on August 19, and there were no trends in adult fish weights, lengths, survival, or gonad weights that could be attributed to the effects of zinc.

The variation in female mortality from tank to tank probably indicates some variation in aggressiveness from male to male, and variation in aggressiveness among males may explain why there were some tanks at all concentrations where no spawning occurred (Table 13). For example, the male in tank 17 (a tank receiving .025 mg/l zinc) killed, or contributed to the death of eight females in succession, and no spawning ever occurred in this tank. Once a spawning did occur in a tank, it was highly likely that several more would occur. In contrast to the multiple spawnings obtained in the control tanks and tanks receiving zinc concentrations of .075 and .025 mg/l, however, only a single spawning in one tank occurred

Table 11. Zinc and dissolved oxygen concentrations, and temperatures in breeding tanks

		Measured zinc concentrations (mg/l)			D.O. (mg/l)			Temperature (°C)		
Tank	Zn*	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
1	.075	14	.071	.043	5	6.1	0.5	4	30.8	1.0
2	.075	14	.081	.047	5	6.3	0.2	4	31.2	0.5
3	.075	14	.079	.039	5	5.9	0.7	4	31.6	0.5
4	.075	14	.076	.032	5	6.0	0.7	4	31.6	0.5
5	.075	14	.074	.028	5	5.8	0.5	4	31.6	0.5
6	.250	15	.231	.055	5	5.5	0.6	4	31.0	0.4
7	.250	15	.232	.048	5	5.3	0.7	4	30.6	0.8
8	.250	15	.230	.045	5	5.4	0.7	4	30.6	0.8
9	.250	15	.234	.043	5	5.1	0.9	4	30.1	0.9
10	.250	15	.249	.044	5	5.3	0.8	4	30.1	0.8
11	.000	14	.028	.023	5	5.5	0.6	4	30.5	0.9
12	.000	14	.019	.007	5	5.7	0.5	4	30.5	0.6
13	.000	14	.019	.010	5	5.5	0.6	4	30.5	0.7
14	.000	14	.020	.014	5	5.7	0.5	4	30.5	0.9
15	.000	14	.017	.011	5	5.4	0.8	4	30.4	0.9
16	.025	14	.028	.012	5	5.6	0.7	4	30.5	0.6
17	.025	14	.040	.019	5	5.4	0.7	4	30.4	0.5
18	.025	14	.041	.018	5	5.8	0.5	4	30.4	0.5
19	.025	14	.035	.017	5	5.7	0.5	4	30.4	0.8
20	.025	14	.033	.012	5	5.4	0.7	4	29.9	0.5

* Nominal zinc concentrations (mg/l)

N-number of readings

Table 12. Survival of adult bluegills and weights, lengths, and condition of gonads of adults at end of breeding experiment

Tank	Zn (mg/l)	[a]No. males removed before end of experiment	[a]No. females removed before end of experiment	Wts. (gms)	Standard lengths (cm)	Males No.
1	.071	0	*3	61.8	11.5	1
2	.081	0	4	69.6	11.5	1
3	.079	*1	0	48.2	10.5	1
4	.076	0	*1	60.9	11.6	1
5	.074	0	1	37.6	10.2	1
6	.231	0	5	69.7	12.1	1
7	.232	0	0	71.7	12.0	1
8	.230	0	2	34.0	10.0	1
9	.234	0	3	62.7	11.7	1
10	.249	0	0	26.1	8.9	1
11	.028	0	4	61.3	11.7	1
12	.019	1	1			0
13	.019	0	3	78.4	11.9	1
14	.020	0	*2	67.6	12.1	1
15	.017	0	*2	72.6	12.3	1
16	.028	0	0	72.5	12.3	1
17	.040	0	*9	70.0	11.8	1
18	.041	0	2	93.4	12.9	1
19	.035	0	2	84.8	13.0	1
20	.033	0	5	31.6	9.6	1

*One fish removed because eye was diseased. No more than one fish per tank contracted this disease.

[a]Fish with eye disease, dead fish, and fish that had lost their equilibrium were removed immediately. A ratio of three females and one male was maintained per tank by replacement of fish until July 20, 1971.

[b]Mean values (with ranges in parentheses) are shown for tanks containing more than one female.

Fish removed at end of experiment						
		Females[b]				
Gonads		Wts.	Standard		Gonads	
Wt(gms)	Condition[c]	(gms)	lengths(cm)	No.	Wt(gms)	Condition[c]
1.2	R	13.1	7.2	1	1.6	1R
1.5				0		
0.9		24.2(16.6-33.6)	9.1(8.5-9.8)	3	1.6(0.1-2.6)	2R, 1N
1.7		31.2(23.0-39.4)	9.6(8.9-10.2)	2	2.3(1.3-3.3)	2R
< .1	N	29.4(23.7-35.0)	9.6(9.4-9.7)	2	2.8(1.5-4.1)	2R
1.0				0		
1.2		29.1(22.0-35.4)	9.4(8.3-10.0)	3	1.3(0.5-2.2)	3R
0.2		44.6(38.3-51.0)	10.4(10.0-10.8)	2	2.7(0.6-4.8)	1R, 1N
0.9	R	35.2(29.2-41.3)	9.9(9.5-10.3)	2	2.4(2.3-2.6)	2R
0.5		36.2(25.2-46.4)	9.7(9.0-10.3)	3	3.2(2.4-4.1)	3R
0.8	R			0		
		31.7(24.6-35.2)	9.5(9.0-9.8)	4	2.6(2.0-3.3)	4R[d]
0.9	R	38.4(34.2-42.7)	10.1(9.6-10.6)	2	3.0(3.0-3.1)	2R
1.6		39.1	10.3	1	1.7	1R
1.0	R	21.6	8.7	1	0.6	1R
0.8	R	28.6(28.1-29.0)	9.5(9.3-9.7)	2	1.6(1.3-1.9)	2R
2.1	R			0		
1.0	R	13.4	7.7	1	0.1	1N
1.0		26.8	9.3	1	2.9	1R
< .1	N	20.6(7.8-32.1)	8.5(6.6-9.7)	4	1.6(0.4-3.5)	4R[d]

[c]R = ripe, and indicates that milt was extruded when the sides of the males were squeezed; or in the case of females, that the ovaries were swollen and filled with pinhead-size eggs. N = not ripe, and indicates that the testes weighed less than .1 gm and no milt was extruded from males; in the case of females, no eggs were visible. The number of ripe and unripe females in each tank is shown.

[d]An extra female was added to tanks 11 and 20.

at a concentration of .250 mg/l zinc (Table 13). Since eggs or milt could be extruded from all the breeders at the beginning of the experiment, and since most of the fish had ripe gonads when the experiment terminated, the results in Table 13 indicate that a zinc concentration of .250 inhibits spawning in ripe fish.

Table 13 also shows the percentage hatch in each tank. Where more than one hatching was used, the mean percentage hatch is shown, with the range in parentheses. Hatching data from eight spawnings were not used because some of the eggs were hatching in the breeding tanks in less than 24 hours at temperatures of 30-31°C. Attempts to remove eggs from these nests by pipetting generally caused the egg membranes to rupture, releasing the fry. In these cases, the percentage hatch in 48 hours of the eggs that were transferred without rupturing was confounded with the 48-hour survival of the fry that were also unavoidably transferred to the egg hatching cups. A hatch of 21% from one spawning in tank 16 that was heavily fungused was also excluded. The hatch obtained from the one spawning at the highest zinc concentration was low (43%), but within the range of values in the other zinc concentrations.

The number of fry introduced to the old rearing chambers with 0.8 mm mesh outlets and the number introduced to the modified chambers with smaller mesh outlets are shown in Table 14. The introductions are shown in chronological sequence from left to right across the rows. For example, two introductions of fry were made to rearing chamber 4: one introduction of 50 fry from tank 4, and a later introduction of 51 fry from tank 1, after inspection revealed that there were no survivors from the first introduction. Growth and survival data in the right-hand portion of the table were always obtained on fish from the last introduction reported in the left side of the table. Survival of young bluegills at all zinc concentrations was poor. Mortality was highest during the first weeks, and can probably be attributed to starvation. The plankton collections that were fed to the young bluegills starting August 24 were never very rich, and the powdered baby fish food may not have been utilized. Once the young were large enough to feed on brine shrimp, survival improved as shown by the reduction in mortality between 30, 60 and 90 days as compared to the mortality between day 0 and day 30. At a zinc concentration of .250 mg/l, however, no bluegills survived longer than 30 days. Fry obtained from eggs spawned in a zinc concentration of .250 mg/l and fry obtained from eggs spawned in other zinc concentrations were placed in .250 mg/l zinc. Most of the fry from all these sources died within three days in 250 mg/l zinc and were visible on the bottom of the rearing chambers. In addition, fry taken from .250 mg/l zinc and placed in control tank 11 showed poor survival: only four fish survived for 30 days, one for 60 days, and none for 90 days.

An experiment on the effects on young bluegill of momentary exposure to a high zinc concentration was inadvertently conducted when a hose separated from a connector and fell into rearing chamber 5 while a stock bottle was being filled with concentrated zinc solution. The

Table 13. Spawning of adult bluegills and percentage hatch of eggs at four zinc concentrations

Tank	Mean zinc concentration	Total no. of eggs spawned[a]	Total no. of spawnings	Mean no. of eggs per spawning[a]	Percentage hatch[a, b]
1	.071	8414	3	2805	72 (71-72)
2	.081	0	0	0	0
3	.079	10647	8	1331	66 (49-78)
4	.076	4736	5	1184	57 (44-68)
5	.074	0	0	0	0
6	.231	0	0	0	0
7	.232	0	0	0	0
8	.230	0	0	0	0
9	.234	1009	1	1009	43
10	.249	0	0	0	0
11	.028	7188	4	1797	62 (35-76)
12	.019	0	0	0	0
13	.019	0	0	0	0
14	.020	227	2	114	33
15	.017	5849	7	985	73 (47-96)
16	.028	4274	3	1425	86
17	.040	0	0	0	
18	.041	0	0	0	
19	.035	10202	5	2040	78 (65-90)
20	.033	0	0	0	

[a]Number of eggs and percentage hatch were not determined for all spawnings because of premature hatching, fungus infestation, etc. (see text).

[b]Where more than one hatch was used, the mean percentage hatch is shown, with the range in parentheses.

Table 14. Survival and growth of bluegills in four zinc concentrations

Rearing chamber	Zn mg/l	No. of fry introduced to old rearing chambers	No. of fry introduced to new rearing chambers	Mean total lengths (mm) Days 30	60	90
1	.071	50[1][a]	50[1]	21.8(5)[b]	28.8(5)	38.8(5)
2	.081		50[4]	13.2(4)	20.3(3)	34.3(3)
3	.079	50[3]	50[3],45[19]	(0)	(0)	(0)
4	.076	50[4]	51[1]	21.0(4)	24.5(4)	36.6(4)
5	.074		50[3]	12.2(19)[c]	19.2(18)	26.9(14)
6	.231	50[15],51[15]	53[9]	(0)	(0)	(0)
7	.232		58[9]	(0)	(0)	(0)
8	.230	50[11]	50[1],58[19]	(0)	(0)	(0)
9	.234	50[11]	51[9]	(0)	(0)	(0)
10	.249	50[15]		(0)	(0)	(0)
11	.028	50[11]	50[11],51[9]	6.8(4)	10.0(1)	(0)
12	.019					
13	.019					
14	.020		50[15],40[14]	9.5(2)	21.2(2)	27.8(2)
15	.017	50[15]		- - -[d]	20.0(2)	33.0(1)
16	.028	50[16]	56[16]	(0)	(0)	(0)
17	.040					
18	.041		51[19]	(0)	(0)	(0)
19	.035	50[19]	50[19]	14.4(7)	19.3(6)	30.6(6)
20	.033					

[a] Numbers in brackets indicate the number of the tank where the fry were obtained.
[b] Numbers in parentheses indicate the number of fish.
[c] At least 2 fish died as a result of a zinc spill on day 22 (see text).
[d] The fish in chamber 15 were not counted or weighed on day 30.

hose was removed from the tank in a fraction of a second and the young bluegill were transferred to the proper zinc concentration within two minutes, but two of the 21 fish died within 15 minutes. During the two minute period, the 22-day-old bluegills had been exposed to a zinc concentration of 9.18 mg/l, although the concentration initially may have been higher in portions of the chamber. There were 19 survivors on day 30, 18 on day 60, and 14 on day 90. After the initial deaths, survival in chamber 5 was comparable to survival in other tanks at the same concentrations.

In summary, it appears that a zinc concentration of .250 mg/l inhibits spawning in adult bluegills brought into breeding condition in dechlorinated municipal water containing no added zinc, and causes complete mortality of bluegill fry. Water containing no added zinc and zinc concentrations of .075 and .025 mg/l do not have these effects.

The lowest zinc concentration tested in the apparatus for monitoring fish breathing was 2.55 mg/l. This concentration was detected by the monitoring method, and the reproduction and growth experiment shows that 1/100 or 1/34 of this concentration might be safe for chronic exposure of bluegills, but that 1/10 of this concentration certainly is not.

SECTION VI

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SECTION VIII

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Sparks, R. E., W. T. Waller, J. Cairns, Jr. and A. G. Heath. 1970. Diurnal variation in the behavior and physiology of bluegills (Lepomis macrochirus Rafinesque). The ASB Bulletin. 17(3): 90 (Abstract).

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Cairns, J., Jr., R. E. Sparks and W. T. Waller. 1971. (manuscript in press) The use of fish as sensors in industrial waste lines to prevent fish kills.

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The presence of zinc at concentrations of 8.7, 5.22, 4.16 and 2.55 mg/l in dechlorinated municipal tapwater was detected by an increase in breathing rate or a change in breathing rate variance of bluegills. None of the fish exposed to the three lower concentrations died during the experiments. The criterion for detection was an arbitrary number of responses occurring at the same time. When the criterion was changed from a single response to three responses occurring at the same time, the number of false detections ("detections" occurring before zinc addition) decreased, but the lag between zinc addition and detection increased. Zinc concentrations of .025 and .075 mg/l (approximately 1/100 and 1/34 of 2.55 mg/l, respectively) did not appear to affect the reproduction and growth of bluegills in the laboratory, but .250 mg/l zinc (approximately 1/10 of 2.55 mg/l) inhibited spawning in ripe bluegills and killed newly-hatched fry. An in-plant system for the prevention of fish kills caused by spills could be developed by monitoring several biological functions of fish simultaneously to obtain informational redundancy and reduce error; by exposing test fish to higher waste concentrations than occur in the receiving stream as a safety factor; automating the collection and analysis of data to reduce lag time; and by choosing appropriate criteria for detection.

17a. Descriptors
*Water pollution control, *Industrial wastes, *Bioindicators, Fishkill, Fish physiology

17b. Identifiers
*Biological monitoring, Bluegill, Zinc, Lepomis macrochirus Rafinesque

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